SPECTROPHOTOMETRY OF LIVING HUMAN SKIN IN THE ULTRAVIOLET RANGE*

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INTRODUCTION

Spectrophotometry of living human skin carried out in the range of the visible spectrum by means of the Hardy recording spectrophotometer (1) has indicated the desirability of extending this type of examination into the ultraviolet range. Recently an instrument was devised by one of us (N. A. F.) which, despite certain limitations noted below, was useful for this purpose (2). The results so far obtained by its use are promising in the identification of substances in the skin known to exhibit absorption bands in the ultraviolet region. It seems desirable to publish this preliminary report to indicate the possibilities of the method.

Attention will be paid primarily to the spectrophotometry of *in vitro* solutions of constituents of the skin, although several curves derived from examination of the skin of living subjects will be presented. These *in vitro* examinations are essential at the outset of such a study, because one must be familiar with the absorption bands of individual components in order to identify them in the curves obtained from the actual skin. It has been found that the light reflected from the skin which produces the curves, is influenced by substances belonging specifically to the dermis, epidermis, and subcutaneous tissue—such as melanin, melanoid and carotene, as well as by others present in the blood circulating through the skin—such as oxy- and reduced hemoglobin. Because of the multiplicity of substances carried in the plasma, this fluid was separately examined *in vitro*.

In previous work in the visible zone, scattering had been shown to play a considerable part in the nature of the reflectance from the skin. No study was attempted of the effect of this optical phenomenon on spectrophotometry in the present range.

Since the main object of the method is to facilitate the identification of individual components, the results will be presented with reference to the spectrophotometric characteristics of various substances *in vitro*, and their subsequent identification, where possible, in living skin.

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MATERIALS AND METHODS

Instrument and procedure. The instrument is a Harrison recording spectrophotometer (3) modified through the addition of an ultraviolet, continuous spectrum light-source, and an ultraviolet integrating sphere. The spectrophotometer as originally designed was suitable only for transmission measurements and did not provide a continuous spectrum source in the ultraviolet region. The band width of the incident beam at any given instant is 2 mμ. A continuous reading of the transmission as a function of the wave length is made in $3\frac{1}{2}$ minutes. The absorption in certain regions of the spectrum characteristic for each substance, and known as its absorption band, is recognized as a localized lowering of the transmission reading. The depth of the band is proportional to the concentration of the substance in solution.

Considerable care was taken in the choice of *solvents*, for many substances colorless to the eye have pronounced absorption bands in the ultraviolet region, which might be confused with bands of the substances under study.

Spectrophotometric examination of the intact skin is performed through reflectance determinations. In this case the incident light is directed upon the area to be examined, and the measurement is made of the ratio of reflected to incident intensity. The intensity of irradiation and period of exposure by any given band width are so small as to have no actinic or heating effect. The rapidity, lack of discomfort, and objectivity, of the method makes it quite suitable for examination of human subjects.

The identification of a given substance in the skin is possible through the recognition of the characteristic band or bands in the spectrophotometric curve. The depth of each band is proportional to the quantity of the substance present.

Limitations of the Instrument. The Harrison spectrophotometer records its data automatically in the form of 2000 points across the spectral range from 240 to 600 mμ. Because of electrical "noise" inherent in the instrument these points do not fall along a perfectly smooth curve but vary randomly about it with an average spread which increases with decreasing light from the sample. Thus, the spread of points was more than five times greater in the case of reflectance curves than in the case of transmittance curves because of light lost in the integrating sphere. The smooth curves shown in this paper were drawn through the points by hand on the basis of a visual average of the position of the points. In the case of transmittance curves the spread of the points was so slight that the curve was clearly defined and the reproducibility of repeated measurements was usually better than $\pm 1\%$ of absolute value, for example $\pm 0.2\%$ at 20% transmittance. In the case of reflectance measurements where the spread was greater the *average* position of the curve could be located nearly as precisely as in the case of transmittance measurements but there was some uncertainty regarding the fine details. By inspecting several curves of the same subject or type of subject a decision could often be reached regarding the validity of an apparent feature of a given curve. It was determined to indicate in the drawn curve only such absorption bands as were unambiguously indicated. Where there was question as to the veracity of a feature of the curve shown by the

points, the curves shown in this paper have been drawn with that feature omitted. There may be, therefore, fine structure in the reflectance curves which we have not reported, but we feel confident that any absorption bands shown by our published curves are real. Obviously, further research in this field should not neglect the improvement of spectrophotometric equipment so that additional absorption bands, if they exist, may be found. The need is for greater photometric precision, the wavelength resolving power of the Harrison instrument being well in excess of the requirements of our work.

Substances tested in vitro. The pigments which exhibit absorption bands in the visible range were restudied. These include those which may be said to reside within the skin structure proper, namely: *melanin*, *melanoid* and *carotene*; and those which are present by virtue of the constant perfusion of the cutaneous blood vessels, namely: *oxy-hemoglobin*, *reduced hemoglobin*, and pigments of the *blood plasma*. Of the many materials which show absorption in the ultraviolet alone, only *vitamin-A* has so far been identified.

The *melanin* was prepared by maceration of a segment of skin from a negro cadaver, according to the method of Abel and Davis (4). It was examined in various dilutions in 5% KOH. We attempted to produce melanoid by treating the solution of melanin with sodium hydrosulphite (5), but further work will be necessary to clearly identify the resultant substance as melanoid. A sample of crystalline *carotene* (General Biochemicals, Inc.) consisting of 90% beta, and 10% alpha, was examined in a solution of petroleum ether. Although the composition of lipochromes in the body is varied, beta carotene is probably one form of these pigments present in greatest amount. Human blood in distilled water was used as an *oxy-hemoglobin* solution. The assumption made, that the extreme dilution adequately minimized the characteristics of the other materials present in blood, was verified by obtaining parallel results from purified human and bovine hemoglobin. *Reduced hemoglobin* was obtained by the addition of sodium hyposulphite ($(Na_2S_2O_4)$). Comment on the effect of this salt on the spectrophotometric reading will be made below. Synthetic *Vitamin A* was kindly supplied by Prof. Nicholas Milas of the Massachusetts Institute of Technology. Seven samples of human *plasma* were obtained from the Massachusetts State Blood Laboratory through the kindness of Dr. H. J. Banton.

Subjects Examined. These included 2 men, a woman, and a boy of Caucasian stock; and 1 Negro, 1 Mulatto, 1 East Indian and 1 Filipino, all of whom were males. Readings were made on an average of 8 regions of each subject.

RESULTS

Melanin: The transmittance curve of melanin shows an absorption gradually increasing from the red end of the spectrum to the violet. In the ultraviolet, the absorption continues to increase steadily. No demarcated band was found in the range studied, from 240 to 600 mμ (Fig. 1). The influence of a varying quantity of melanin in the skin upon the reflectance curves is indicated in Fig. 2. The presence of much melanin blunts the absorption bands of other pigments in the visible spectrum. This is especially noticeable in the curves obtained from people of the very dark races. It was hoped that this effect might not prove to be so pronounced in some part of the ultraviolet range. Unfortunately, it is apparent that melanin masks other materials in the ultraviolet as effectively as it does in the visible portions of the spectrum.

Melanoid: Spectrophotometry in the visible region had revealed a cutaneous

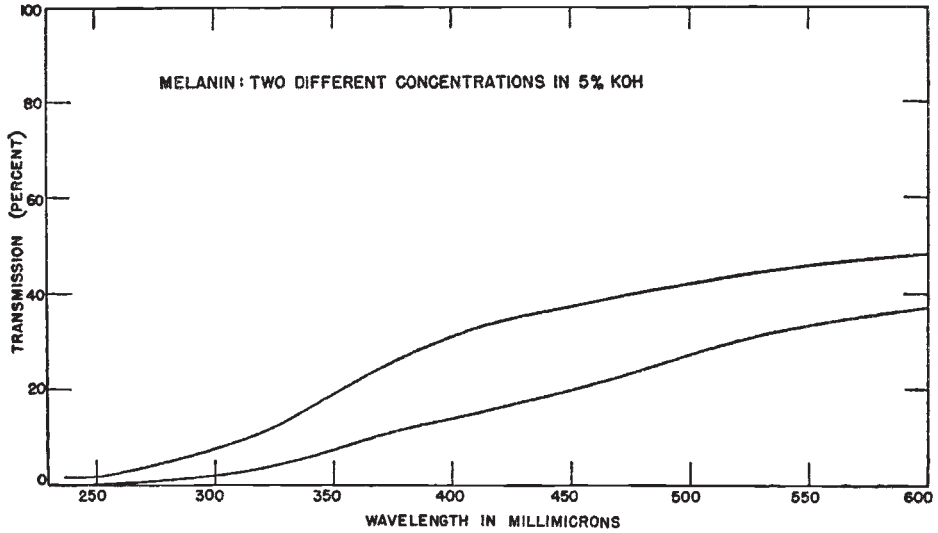

FIG. 1. The transmission of melanin in 5% KOH solution. The lower curve is of a more concentrated solution of melanin producing greater absorption and therefore less transmission than the weaker concentration. There is no demarcated band, but rather a gradually increasing absorption from the red end of the spectrum at 600 mμ into the ultraviolet.

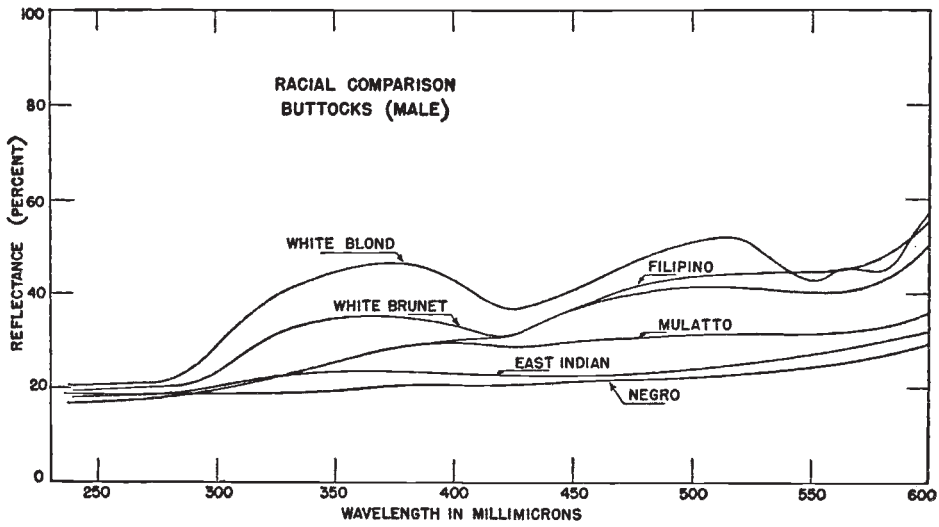

FIG. 2. The role of melanin in skin color of different races. The region of the buttocks was chosen as one least affected by environmental factors. The difference in color and spectrophotometric curve is related only to melanin concentration. The variability in hemoglobin content of the lighter subjects is entirely fortuitous. In the darker subjects the larger quantity of melanin obscures the characteristics of other skin pigments.

pigment which we named melanoid, inasmuch as it appeared to be a breakdown product of melanin. Its presence had been indicated by a flattening of the skin

reflectance curve in the vicinity of 400 mμ, the lower limit of the measurements. In the present study the production of melanoid was attempted by the addition of sodium hydrosulphite ($NaHSO_2$) to melanin (5). The spectrophotometric curve of the resultant material showed a sharply increasing absorption from 415 mμ into the near ultraviolet, much greater than that exhibited by melanin in this zone. Further work will be necessary before positive identification of the material as melanoid will be possible.

Carotene. Figure 3 shows the spectrum of a mixture of *beta* and *alpha* carotene in a solution of petroleum ether. Of the several bands noted, only one, that at 485 mμ, which shifts in skin to 482 mμ, was surely identified in readings of the intact skin (Fig. 4).

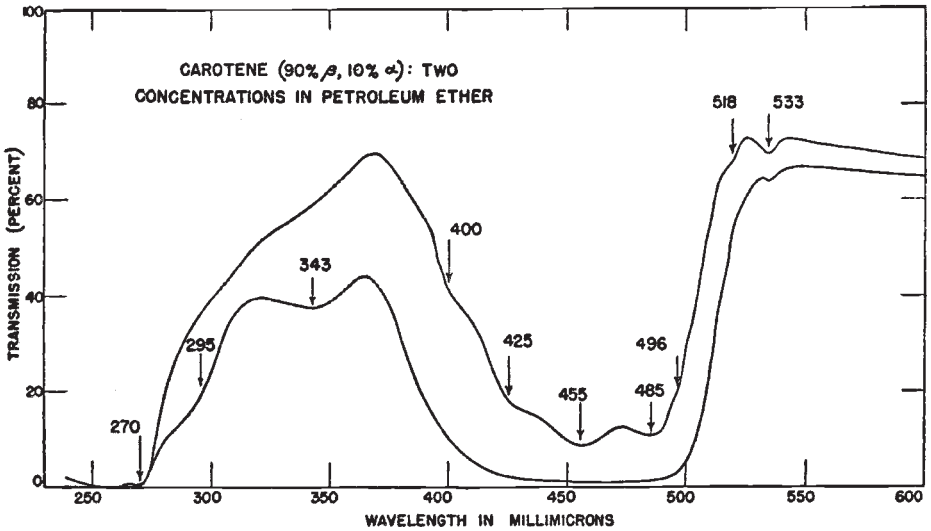

FIG. 3. The transmission of carotene in petroleum ether. The characteristic absorption bands are indicated. Only the band at 485 mμ, which shifts to 482 in the skin, can be identified in the curves of the living subject.

This represents neither an advantage nor a disadvantage over spectrophotometry in the visible range, by which method the same band could be easily found. Indeed under certain conditions the band at 455 mμ, was also seen (1c). According to Miller (6), these 2 bands are among those of *beta* carotene. Of the several additional bands that exist for this material, those at 518 and 524 mμ, were indicated weakly in the examination, with the Hardy instrument, of an extract of subcutaneous fat, but could not be identified in the curves of intact skin, perhaps because of confusion by the absorption of hemoglobin.

The absorption effect of carotene and other lipochromes in blood plasma will be mentioned below (Fig. 9).

Hemoglobin and Oxy-hemoglobin. Figure 5 shows the spectrum of greatly diluted hemolyzed blood. Comparison with curves of solutions of pure crystallized hemoglobin shows that the effects of other blood pigments in normal indi-

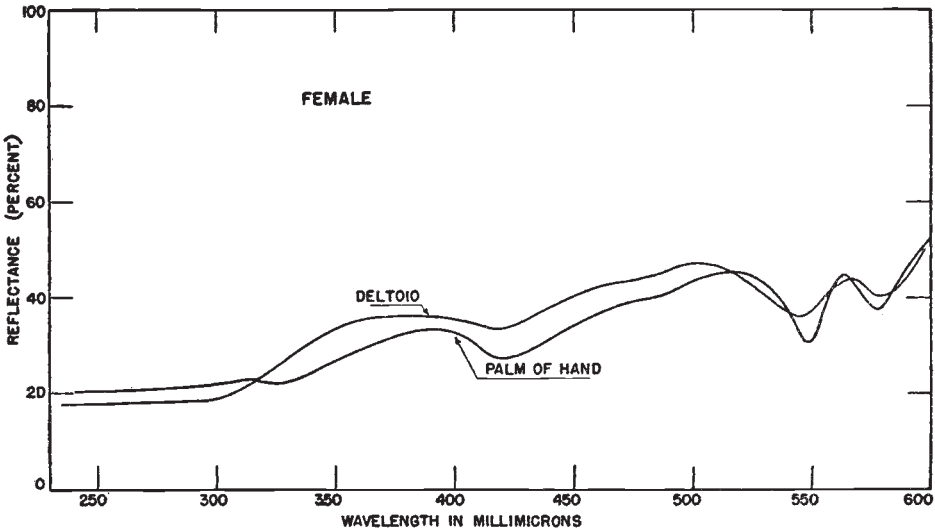

FIG. 4. The identification in skin curves of oxy-hemoglobin, carotene, and vitamin A. The curve of the deltoid shows more melanin, as indicated by the depression in the extreme ultra-violet. The palm curve is lower in almost all the rest of the spectrum, because of its higher hemoglobin content, which is also better oxygenated, as indicated by the more pronounced absorption at 578, 548, and 417 mμ. Both curves show a strong absorption band at 482 mμ, characteristic of carotene, a substance more abundant in the female than in the male. The absorption of vitamin A at 325 mμ is easily seen in the curve of the palm.

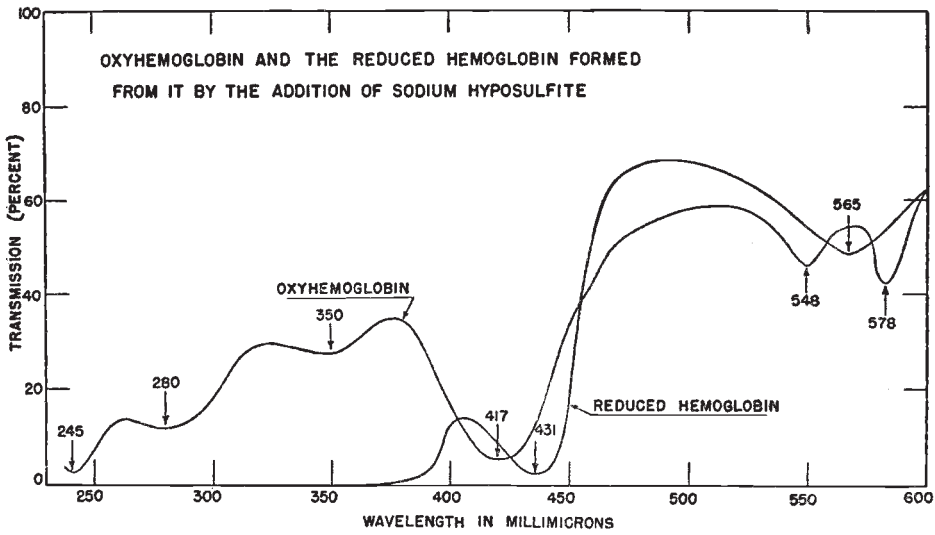

FIG. 5. The transmission of oxy- and reduced hemoglobin as seen in greatly diluted hemolyzed blood. Of the oxy-hemoglobin bands, only those at 548 and 578 mμ are sharply demarcated in readings of the skin, although that at 417 may also be seen. The reading of reduced hemoglobin is abridged by virtue of the added absorption of the sodium hyposulphate. In other determinations we have observed additional absorption bands for reduced hemoglobin at 260 mμ, with a flexion point in the curve about 335 mμ (see fig. 6). In readings of the skin the band at 565 mμ may be clearly seen, that at 431 somewhat less distinctly.

viduals is negligible, and the curves may therefore be considered as that of oxyhemoglobin proper. Absorption bands are seen at 578, 548, 417, 350, 280 and 245 mμ. Those in the visible range are shifted slightly from their position as determined with the Hardy spectrophotometer. This is probably related to the narrower slit width of 2 mμ in the present spectrophotometer, as compared with the greater width of 10 mμ in the Hardy instrument.

The bands at 578 and 548 mμ can be seen in skin curves from regions of active blood flow (heel, fig. 7), as previously noted in spectrophotometry in the visible

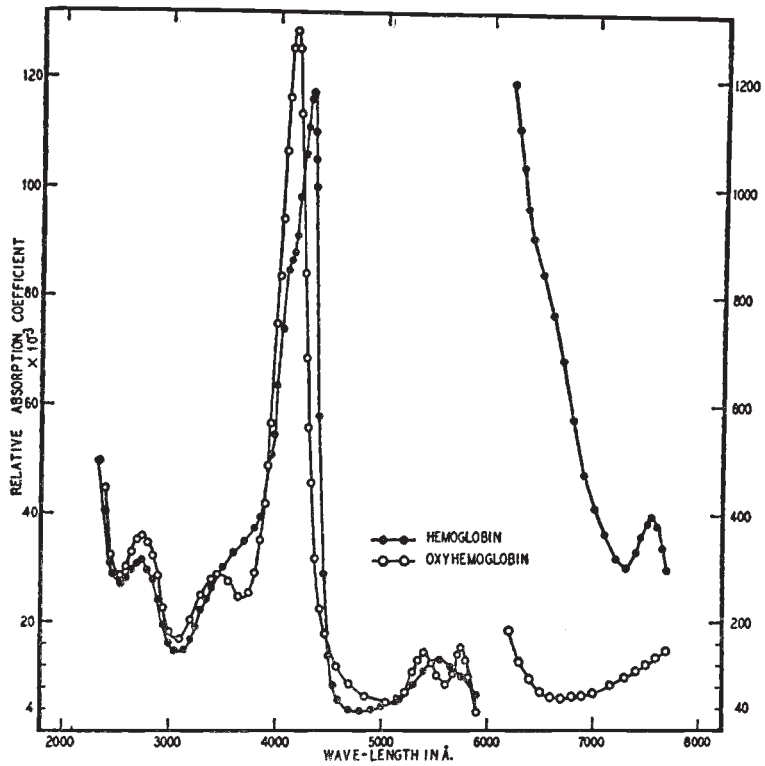

FIG. 6. The absorption spectra of oxy- and (reduced) hemoglobin from Sidwell et a (7) Note that the curves are plotted as the inverse of the transmission shown in the othe figures. The hemoglobin was reduced by tonometric means, obviating the added absorpr tion in the ultraviolet of the reducing agent used in the current study. (From the J. Biol. Chem. **123:** 335, 1938. With permission of the authors and publishers.)

range. None of the bands in the ultraviolet have been sufficiently pronounced to be identified in the readings of the skin. Presumably this is due to the masking effect of melanin.

Reduced hemoglobin was produced by the addition of $Na_2S_2O_4$ to the blood solutions. In the visible parts of the spectrum, bands previously identified were again seen at 565 and 431 mμ. This represents a slight shift from the measurements with the Hardy instrument. At the dilution used in figure 5, the transmission falls to zero below 370 mμ. On further experiment, it was determined that $Na_2S_2O_4$ itself, while colorless in the visible range, has strong absorption in the

ultraviolet, and can account for much of the apparent absorption of hemoglobin when reduced in this fashion. By varying the quantities of the sodium hyposulphite and blood, it was possible to obtain absorption curves in the ultraviolet that appeared valid for the reduced hemoglobin proper. In these curves an absorption band can be seen at 260 mμ, and a flexion point in the curve about 335 mμ. This correlates well with the values obtained by Sidwell et al. (7), using hemoglobin reduced by tonometric means (Fig. 6). (Their curves show an absorption band at 275 mμ, and a flexion point about 340 mμ.)

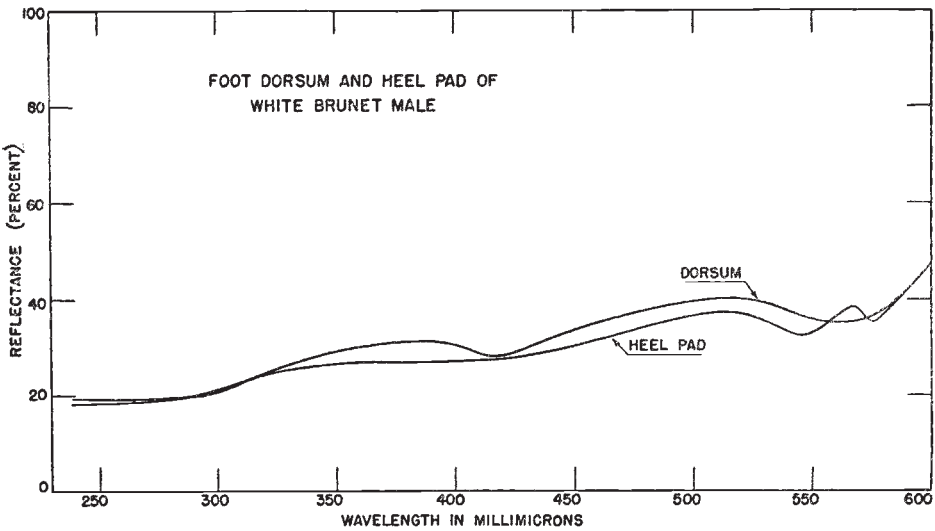

FIG. 7. The contrast in skin curves caused by differences in hemoglobin content and oxygenation, and the influence of melanin and melanoid. The dorsum contains less hemoglobin, which is also less well oxygenated, with consequent prominence of the band of reduced hemoglobin at 565. The expected high levels of reflectance at other areas are flattened through the absorption of melanin.

The curve of the heel pad indicates a more active blood flow, with marked oxy-hemoglobin bands at 578 and 548 mμ. The 417 mμ band of oxy-hemoglobin is at least partly responsible for the gradual depression in the violet, but this is almost certainly accentuated by the absorption of melanoid. Melanoid rather than melanin is probably the major factor in the blunting of the entire curve, for much melanin would not allow the elevation about 350 mμ. Some of the elevation of the curve in the extreme ultra-violet may be caused by scattering.

The effect on the skin curve of a predominance of one form of the hemoglobin over the other, can be seen in the comparison of the curves of the dorsum and of the heel pad of the foot (Fig. 7). The reflectance of the dorsum of the foot—an extensively venous region with relatively sluggish blood flow—gives a strong indication of reduced hemoglobin in the visible range. The heel pad—a highly arterialized area—shows strikingly the presence of oxy-hemoglobin.

Vitamin A: This is so far the only material with no absorption in the visible range, which we have identified in readings of the skin. Vitamin A is known to show an absorption band at 325 mμ (8) (Fig. 8). This band was apparent in the

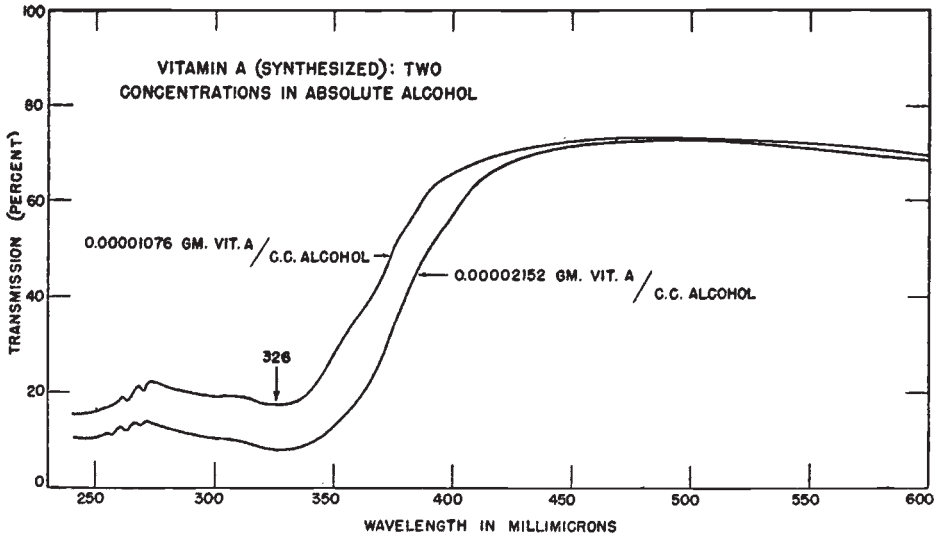

FIG. 8. The transmission of vitamin A in alcohol. The characteristic absorption band is seen at 326 mμ. This is shifted 1 mμ from its usual location at 325, perhaps because of the source of this particular sample. The significance of the fine structure between 255 and 270 mμ has not been determined.

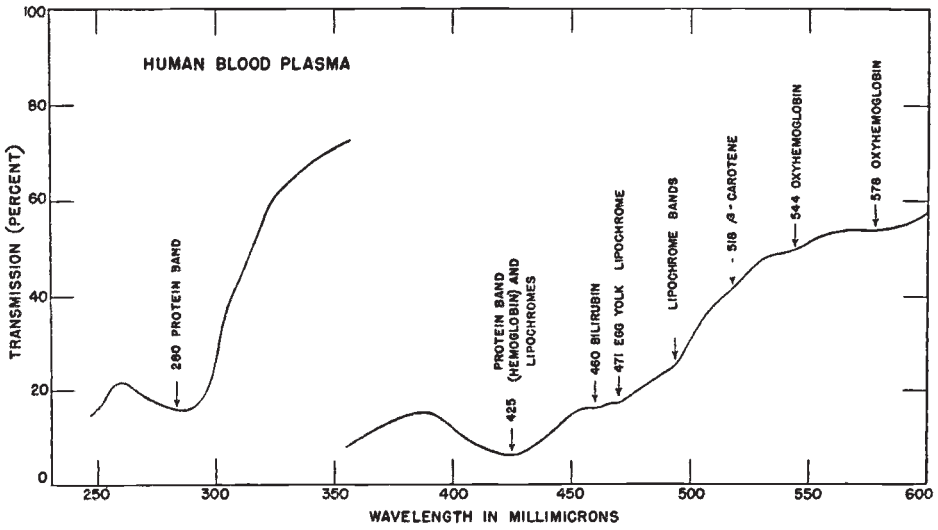

FIG. 9. The transmission of diluted blood plasma. The curve on the left represents a greater dilution of the same sample as that used on the right. The absorption of various constituents is indicated.

curves of the palm in the female subject (Fig. 4) as well as in that of the white adult male. We not know whether this substance was present in the skin structure proper, or in the blood.

Blood Plasma. Figure 9 shows the transmission curve of human plasma. The

part played by the absorption of its constituents is indicated where known. Substances imparting color to normal plasma, and therefore detectable in the visible portions of the spectrum, include bilirubin, carotenoids and oxy-hemoglobin. The different samples of plasma varied considerably in the quantity of each of these substances.

Oxy-hemoglobin can be dismissed briefly as an impurity accidentally produced in the separation of blood. Undoubtedly it normally plays no part as a plasma constituent in the living body. Bilirubin has its characteristic band at 460 mμ, with strong absorption in the region below 300 mμ. Of the lipochromes, the bands of carotene have been noted by previous workers. Heilmeyer (9) found it difficult to detect in plasma the bands pertaining to individual forms of carotene, but believed they were responsible in the aggregate for a large area of absorption between 520 and 440 mμ, and again between 440 and 420 mμ. Under conditions of high dietary intake, he could also note the bands of certain xanthophylls—the luteins of egg yolk and of maize.

A limited study of plasma albumin and globulin indicates that these substances may be separable by ultraviolet spectrophotometry. This brings up the possibility, to be answered in the future, that the proportions of the two may be determined in a sample of plasma, or finally, in the skin.

SUMMARY

An extension of former work on recording spectrophotometry of the skin of living human subjects was attempted by similar measurements extending into the ultraviolet region. The range was 240 to 600 mμ. Readings were made of the reflectance values from various areas of several subjects. In order to aid in understanding the curves of the skin, transmission measurements were made of *in vitro* solutions of substances normally found in integument.

Substances previously identified in the skin by recording spectrophotometry in the visible range were again noted. Of the many substances known to exhibit their characteristic absorption solely in the ultraviolet zone, only one, vitamin A, was so far identified in the skin readings obtained through the reported method.

The method seems promising for the ultimate identification and quantitative determination of additional substances in the living skin. Further technical advances in the photometry involved appear desirable.

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